

REMARKS

1. Definiteness (OA §7); Objections (OA §8)

1.1. Claim 5 was rejected, nominally for lack of antecedent basis for serine in base claim 1, but more properly, if claim 1 indeed did not permit serine at the N-terminal, for failure to further limit the base claim (112/4).

For base claim 1 to read on N-terminal Serine, R19 must be -CH₂OH. Claim 1 said that R19 could be -CH₂X.

Besides being the various specific functionalities "H...substituted heteroalkyl", X may also be a "functional group" (note comma separating it from "heteroalkyl") which is specially defined at P16, L36 to P17, L3. Since this conflicts with the ordinary usage of "functional group", we have amended base claim 1 to define "functional group" with the list from P16, L36 to P17, L3. We have given this an arbitrary label "functional group Q", because it occurs two more times in claim 1.

There is unfortunately, some overlap among the members of the Markush groups. We trust this is not a problem.

1.2. The rejection of claim 8 for reciting a range within a range is moot as that claim has been amended.

1.3. Claims 9 and 10 are said to be drawn to unelected subject matter. This is not a proper basis for an indefiniteness rejection; unelected inventions are withdrawn from consideration.

Moreover, we do not see how claims 9 and 10 can be characterized as drawn to unelected inventions when we elected group I and the examiner defined group I as being claims 1-24 and 27, which of course includes claims 9 and 10. The restriction did not distinguish the subject matter of claims 9 and 10 from that of claim 1.

1.4. Claim 17 has been amended. The wording "an effect on induction of hapten tolerance" has been deleted and instead the wording "the ability to induce hapten tolerance" has been inserted to avoid the term "effect" which the Examiner objected to. Basis may be found at page 4, line 9-10.

1.5. With regard to claim 27, the Examiner says

Claim 27 is rejected wherein the claim is indefinite for whether the fusion protein can contain one copy of one of the possible sequences in claim and then contain several copies of other different sequences of claim 1 or if they are all to be the same or what order they should be or if order and/or sequence matters since the Applicants state in claim 1 that the different sequences comprise one or several different activities listed in claim 1 a)-d). The different possible activities for the different sequences make a fusion protein's activity indefinite.

We have amended claim 27 to recite

A fusion protein comprising at least one sequence corresponding to a compound according to claim 9 where, if it comprises a plurality of such sequences, the sequences may be the same or different.

It is thus clear that there may be more one sequences, and the sequences may be the same or different. There is no teaching in the specification to the effect that order matters.

1.6. Reviewing the specification, we found use of improper language of preference in group I claims 1, 16-18 and 23. Claims 1, 16-18 have been amended, and 23 cancelled. New claims 66-68 are based on the preferred embodiments of claims 16-18.

1.7. The spelling of "vessels", in claim 19 has been corrected.

2. Enablement

2.1. Claims 1-24, 27 and 29 stand rejected for lack of enablement because the specification, while admittedly enabling for "in vitro selectivity studies for MC1 receptors, in vitro capacity to stimulate the second messenger cAMP, and in vitro nitric oxide inhibition [sic, "inhibition]", allegedly "does not reasonably provide enablement for the intended use of any compound of formula I of claim 1 for in vivo use".

The intended in vivo uses questioned by the Examiner are apparently those set forth at office action, page 5, lines 9-11,

i.e.,

in vivo immunomodulatory purposes, amelioration, prevention or inhibition of contact hypersensitivity and edema and inflammation of vessels and vasculitis, blood cell normalization, and inhibition of sensitization of a hapten.

Reviewing the claims, they fall into several categories.

I. Claims 1-8, 24 and 27, as examined, required that the compound optionally possessed at least one of the following properties

- a) showing high affinity for MC1 receptors, and/or
- b) showing high selectivity for MC1 receptors, and/or
- c) showing high capacity to stimulate the second messenger cAMP, and/or,
- d) being an effective inhibitor of NO production.

These activities are not, by their nature, necessarily in vivo, and the Examiner has conceded enablement for these activities exercised in vitro.

However, to put the issue beyond doubt, the optional activity limitation has been excised from claim 1.

II. Claims 9 and 10 did not have any activity or use limitation in the first place.

III. Claims 11 and 12 explicitly require that the compound be capable of activating (11) or blocking (12) MC1 receptors, but these appear to be in vitro activities. Likewise, claim 13 recite inhibiting NO production, an in vitro activity accepted by the Examiner.

IV. Claims 14-22 recite compounds as having one of the activities which the examiner characterizes as an unlikely in vivo activity. Claims 14-22 are now dependent on claim 9. (See also new claims 66-68.)

V. Claim 29 was directed to a prodrug and thus contemplates in vivo use. However, claim 29 has been cancelled.

VI. New claims 69-78 recite one or more in vitro activities, in particular those previously recited in claim 1.

As the Examiner is no doubt aware, while for a method-of-use claim, the recited use must be enabled, for a compound claim, all that is needed is that one disclosed or obvious use be enabled and, if the claim is generic, the enabled use may differ from compound to compound. It is irrelevant to the enablement of a product claim that there are other disclosed utilities which the examiner considers dubious.

It is respectfully submitted that since the Examiner has conceded that the specification is enabling for three in vitro utilities for the compounds of formula I of claim 1, there is no reasonable basis for doubting the enablement of the claims of categories I-III and VI above (i.e., claims 1-13 and new claims 69-78). Category V is moot. Hence, we need only further consider category IV (claims 14-22).

At the outset, we must point out that certain of these claims recite activities which can be manifested in vitro. For example, a compound is immunomodulatory (14) if it stimulates or inhibits T cells. A compound can be said to inhibit contact hypersensitivity (15) in vitro as shown by its effect on Langerhaus cells. Likewise, it can be said to in vitro inhibit sensitization of T cells (16) or to induce T cell tolerance of haptens (17) in vitro. A compound may also affect IL-1, IL-6, TNF, NO, NOS, IL-8 or IL-10 in vitro (21-22). Thus, only claims 18-20 relate to activity at the tissue, organ or organismic level.

Moreover, the category IV claims now depend on claim 9, which specifically recites the peptides MS05 and MS09. The specification discloses working examples of the use of MS05 and/or MS09 in vivo to inhibit sensitization by DNFB (16-17), to inhibit oedema (18), to normalize blood cell counts (20), and to reduce vascular inflammation (19). See P25, L22-P26, L30:

Marked positive treatment effects were found
upon administration of MS05 to BALB/C mice.
Animals were sensitized by injection of

2,4-dinitrofluorobenzene (DNFB). First 30 μ L of 0.5 % DNFB was administered to the shaved abdomen of the mice and after 4 days 10 μ L of 0.3 % DNFB was challenged to one paw, another paw being unchallenged and serving as a control. MS05 was administered, using a solution of MS05 in 0.9 % saline, intraperitoneally two hours before sensitization, and then the same dose was given intra-peritoneally for four consecutive days, each dose of MS05 amounting to 0.05, 0.25, 0.37, 0.5 and 0.75 mg/kg. These treatments with MS05 were found to inhibit paw oedema by 3, 5, 12, 39 and 10 %, respectively, compared to animals that were subjected to the same DNFB administration but which had not been given MS05. Moreover, in these tests the DNFB induced a marked increase in total blood white cell counts as well as marked increase in granulocyte counts of the blood, the increase in total white cell blood counts and granulocyte counts being essentially normalized by the MS05 treatment, the best effect being seen at 0.5 mg/kg of daily intraperitoneally administrations of MS05. The DNFB administration also increased the count of lymphocytes/monocytes in the blood and this increase was essentially returned to normal levels upon the administration of MS05. Blood platelet counts was also increased in DNFB treated animals; the increase being returned towards the normal by administration of MS05. Similar reduction of the oedema induced by DNFB sensitization of the ears of BALB/c mice was seen upon intravenous injections of either MS05 or MS09, using solutions of MS05 and MS09 in 0.9 % saline, to the animals. Thus, these results show that a compound of the invention prevents, ameliorates, and/or inhibits contact hypersensitivity, sensitization by a hapten, and/or has a positive treatment effect on oedema. Moreover these results show that the compound of the invention is capable of normalizing blood cell counts. Therefore the compound of the invention is in a further sense immunomodulatory. Moreover, these results demonstrate the capacity of a compound of the invention to be administered in form of a pharmaceutical.

In further tests the MS05 or MS09 was administered to human microvascular endothelial cells (HMEC-1 cells; Department of Health & Human Services, Centres for Disease Control and Prevention, Atlanta, GA 30333, USA) *in vitro*. The HMEC-1 cells responded with an upregulated expression of mRNA for adhesion molecules ICAM-1, VCAM and E-Selectin, as well as by upregulation of the ICAM-1, VCAM and E-Selectin proteins, upon administration of TNF α (10 ng/mL). The administration of MS05 or MS09 (preferred concentrations of MS05 and MS09 being within 0.01 nM to 10 μ M, and preferred times for contacting the cells with MS05 or MS09 being 3 - 48 hours) led to inhibition of the upregulated expression of mRNA for adhesion molecules ICAM-1, VCAM and E-Selectin, as well as to inhibition of the upregulation of the ICAM-1, VCAM and E-Selectin proteins. These results demonstrate the capacity of the compound of the invention to be immunomodulatory, to be useful for the treatment of inflammation related to the vasculature, e.g. having positive treatment effects in vasculitis. In this context by ICAM-1 is intended intercellular adhesion molecule type 1, by VCAM is intended vascular adhesion molecule, and by E-Selectin is intended endothelial selectin (Sluiter et al, J. Cardiovasc. Pharmacol. 1993, 22 Suppl 4: S37-44; Elangbam et al., Vet. Pathol. 1997 Jan, 34(1): 61-73).

To further support the statement that the compounds of the present invention may be used *in vivo* we have attached a research report showing marked anti-inflammatory effects of one of the compounds MS-05 *in vivo*. In this research report administration, dosage etc. are given.

Conclusively, given the support for *in vivo*-use of the claimed compounds MS-05 and MS-09 in the specification, the predictability for *in vivo*-use in the art and support of *in vivo*-use of one of the claimed compounds in the attached research report, it would not require undue experimentation by one skilled in the art to be able to make and use the claimed invention.

2.2. The enablement rejection (OA §5) of prodrug claim 29

is moot as that claim has been cancelled.

2.3. There is also an enablement rejection (OA §4) of fusion protein claim 27.

While the prior art does not teach a fusion protein comprising one or several copies of the sequence of claim 1, it does teach successful production of hundreds of different fusion proteins in which the fused sequences retain their original activities. Hence, we would say that there is a high level of predictability in the art. Note also that multi domain proteins usually evolve by gene duplication, which implies the existence at an intermediate stage of functional proteins with identical copies of domains.

Moreover, examined claim 27 was calling for fusion, not of disparate proteins from different organisms, but rather of closely related sequences.

Finally, claim 29 has been amended to depend from claim 9, and consequently the claim is not broad; the fused sequences are MS-05 and/or MS-09 sequences.

The Examiner is reminded that the office has repeatedly accepted claims to compounds "comprising" a particular polypeptide sequence of known activity, and this claim simply requires that it comprise a plurality of such sequences.

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3. Miscellaneous

3.1. A substitute power of attorney is enclosed.

Respectfully submitted,

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Evaluation of the anti-inflammatory effect of MS05 in vivo

Female BALB/c mice (weight 20–22 g) were sensitized by treatment of the shaved abdomen with 30 μ l of 0.5% 2,4-dinitrofluorobenzene (DNFB). After 4 day they were challenged with 10 μ l of 0.3 % DNFB to the paw. The unchallenged mice paws served as a control. Twenty-four hours after the last challenge, the difference in paws weight was determined as an indicator of the inflammation (paw edema). Untreated mice were used as time controls

α -MSH treatment

Mice were treated as the control but were additionally injected i.p. with α -MSH (Ser Tyr Ser Met Glu His Phe Arg Trp Gly Lys Pro Val) two hours before sensitization (day 0) and the same dose was administered repeatedly after sensitization during four consecutive days (total dose of α MSH: 2.5 mg/kg). The paw edema inhibition was measured recording the difference in paws weight after twenty-four hours following the last challenge.

MS05 treatment

Mice were treated as the control but were additionally injected i.p. with MS05 (Ser Ser Ile Ile Ser His Phe Arg Trp Gly Lys Pro Val) two hours before sensitization (day 0) and the same dose was administered repeatedly after sensitization during four consecutive days (total dose of MS05: 2.5 mg/kg). The paw edema inhibition was measured recording the difference in paws weight after twenty-four hours following the last challenge.

Groups containing 10 mice each were used for the experiments.

Blood analysis was carried out using the QBC[®] Autoread[™] Plus & QBC[®] Accutube System (Becton Dickinson). In all cases blood samples were collected after twenty-four hours following the last challenge.

Results:

Both α MSH and MS05 treatment significantly inhibited the paw edema with 18% and 39% respectively compared to the control edema group (Edema control: 0.147 ± 0.006 g; α MSH: 0.120 ± 0.002 g; MS05: 0.090 ± 0.004 g).

Moreover, DNFB treatment induced marked leucocytosis, which was prevented by both α MSH or MS05 treatment:

Parameters	Control, untreated mice	Control, edema	α-MSH 2.5 mg/kg	MSO5 2.5 mg/kg
Total White blood cells ($\times 10^9$/L)	4.3	17.5	6.5	4.8
Granulocytes ($\times 10^9$/L)	0.7	8.0	1.6	1.3
Lymphocytes/ Monocytes ($\times 10^9$/L)	3.6	9.5	4.9	3.5